ATTACHMENT A

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BETA-ADRENERGIC RECEPTORS IN AGING RAT BRAIN: MODIFICATIONS INDUCED BY PSYCHOTROPIC DRUGS

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INTRODUCTION

During the processes of development and aging, the organism undergoes several alterations in its biochemical[1-3], physiological[4,5] and pharmacological[6,7] responses to adrenergic stimuli (see review 8). We have been concerned with the age-related changes that occur in the sensitivity of tissues to adrenergic agonists and to drugs that might affect adrenergic function. Our studies have shown that the age-related decline in β-adrenergic responsiveness may be due, at least in part, to a decline in the density of β-adrenergic receptors[9,10]. Using the potent, high affinity β-adrenergic antagonist [^{3}H]dihydroalprenolol (DHA) to specifically label β-adrenergic receptors, we found that the density of these receptors declines significantly with age in several areas of rat brain, including the cerebellum, corpus striatum, cerebral cortex and pineal gland. Since there was no age-related alteration in the apparent affinity of receptors for DHA, or in the IC_{50} value for propranolol in inhibiting specific DHA binding, these studies suggest that the number rather than the properties of the receptors changes with age. The recent reports of similar findings in lymphocytes[11] and erythrocytes[12] suggest that the age-related decline in β-receptors that we observed in brain may be a more general phenomenon associated with senescence.

It is well known that the aging process is associated with a decreased capacity of tissues to maintain homoeostasis under stress or under fluctuating physiological conditions[13,14]. We wondered whether this reduced adaptive capacity might be due to an inability of aged tissue to alter its adrenergic receptors in response to changes in sympathetic input. In young animals it is now clear that adrenergically-innervated tissues compensate for an increased adrenergic input (e.g., prolonged exposure to catecholamine agonists) by decreasing the density of β-adrenergic receptors[8,15-27]. This results in a decreased response of adenylate cyclase to β-adrenergic agonists and a subsensitive physiological response (i.e., tolerance) to these agents[8,15,16,18-20,22-26]. Conversely, reducing sympathetic input to such tissues (e.g., by denervation or by administering drugs that deplete catecholamines or block their actions) causes a compensatory increase in the density of β-adrenergic receptors[8,27,28], an enhanced activation of adenylate cyclase by β-adrenergic agonists and supersensitive physiological adrenergic responses[8,22,24,25,29-33]. Perhaps aged tissues have a decreased ability to make these compensatory changes.

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In the first series of studies to investigate this question we found that when we exposed young rats to light, which decreases sympathetic input to the pineal gland[34] and produces a supersensitive response of adenylate cyclase to norepinephrine[31], there was the predicted increase in β-receptor density in the pineals[10,16,17]. By contrast, there was no significant light-induced increase in receptors in the pineals of 24-month-old rats[10]. These results suggest that aged rats have an impaired ability to increase adrenergic receptors in response to reduced adrenergic input.

The present paper will report the results of our more recent studies of β-adrenergic receptors in aging rat brain. The purpose of these investigations is to provide more information on the relative ability of aged brain tissue to modify its adrenergic receptors in response to chronic changes in adrenergic input. To alter the adrenergic input we have administered chronically various psychoactive drugs known to affect brain adrenergic mechanisms; namely, reserpine, which depletes catecholamines from adrenergic nerve endings, desmethylimipramine (DMI), which increases the amount of catecholamine available for the adrenergic receptor by blocking its reuptake into adrenergic nerve terminals[35,36] and trifluoperazine, which blocks the responses of adrenergic agonists[37-39]. Moreover, since these and many other psychoactive drugs are thought to exert their clinical effects by modifying adrenergic responses we feel these studies might help explain why geriatric patients often respond differently to these drugs.

METHODS

Direct labeling of β-adrenergic receptors in various areas of the brains of Fisher-344 and Sprague-Dawley rats was carried out utilizing DHA as the radioligand in binding assays as described previously[10,40]. In this method specific DHA binding is defined as total binding minus non-specific binding, determined in the presence of excess propranolol which displaces DHA from specific binding sites. From Scatchard analysis of specific DHA binding[41], one can calculate the density of β-adrenergic receptors (B_{max}) and the apparent dissociation constant (K_d) for DHA binding.

RESULTS AND DISCUSSION

Subsensitivity:Effect of DMI Treatment on β-adrenergic Receptors in Rat Brain. In young rats the chronic administration of DMI produces a compensatory reduction in the density of β-adrenergic receptors in certain brain areas[8,19-21]. To determine whether tissues from aged rat brain could develop this same β-adrenergic subsensitivity we studied the effect of DMI on brain beta-receptors of young and old rats. Twenty-four hours following chronic DMI administration to 3-month-old rats (40 μmoles/kg, i.p., twice daily for 3 days) there was a significant

reduction in the number of β-adrenergic receptors in rat cerebral cortex and pineal gland but not in cerebellum[21]. A single acute dose of DMI produced no change in receptor density. The response was not age-dependent, however, in that the response in 24-month-old rats was of the same magnitude as that produced in the younger rats. Our results suggest that the pineal glands and cerebral cortices from aged rats do not differ from those of young rats in their ability to develop a subsensitive β-adrenergic response to increased adrenergic input. This is of interest in view of reports that aged depressed patients do not differ from younger patients in their therapeutic response to tricyclic antidepressants such as DMI[42,43]. Furthermore, the finding that chronic administration of these drugs is necessary to obtain a clinical effect and that chronic treatment of animals with DMI leads to diminished adrenergic reactivity[19,20,26], not increased reactivity, as predicted by the acute effect of the drugs, suggests that one must reevaluate the current theories of depression and the mechanism of action of these agents. At the very least, the consistent finding of a reduced density of β-adrenergic receptors in various brain areas in response to chronic antidepressant treatment should prove to be a valuable tool in the search for new and even more potent, more rapidly-acting antidepressant agents. Perhaps direct-acting β-adrenergic agonists would be useful in providing an even more rapid β-adrenergic subsensitivity and, thus, a more rapid clinical therapeutic effect.

Supersensitivity: a. Effect of Reserpine Treatment on β-Adrenergic Receptors in Rat Brain. In addition to studying the normal physiological variation in β-adrenergic reactivity in pineal gland produced by alternating periods of light and dark (discussed above), we also induced β-adrenergic supersensitivity in rat brain by the chronic administration of reserpine. This drug, by causing a long-term depletion of catecholamines, produces an enhanced activation of brain adenylate cyclase by β-adrenergic agonists[29,33,44-48]. We found[8,28] that administration of reserpine daily for 3 days to 3-month-old rats produced dose-dependent, statistically significant increases in specific DHA binding in pineal gland (Figure 1) and cerebral cortex at doses as low as 0.25 μmoles/kg, i.p., and in cerebellum, at 1 μmole/kg. No increase in DHA binding was observed, however, in these brain areas 30 minutes following an acute dose of the drug. This suggests that the chronic effect of decreased noradrenergic input, rather than the acute presence of the drug, is important in increasing DHA binding. To determine whether the increased DHA binding produced by reserpine was due to an increased density or affinity of the receptors, we carried out Scatchard analyses of DHA binding in cerebral cortices from 3-month-old rats. Reserpine did not change in the K_d for DHA binding (12 ± 1nM) but did produce a significant

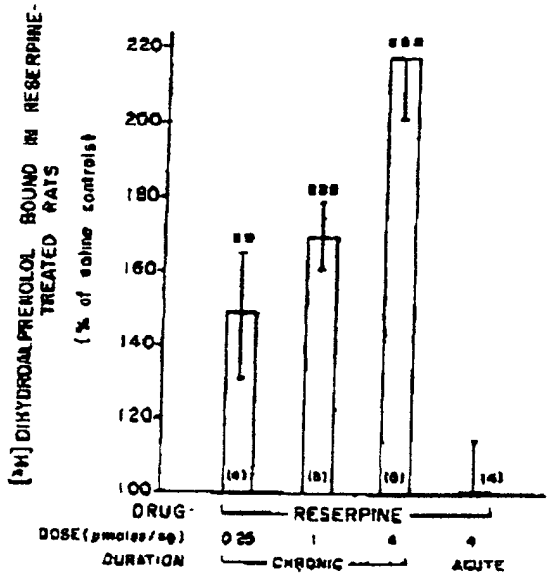

Figure 1. Effect of chronic reserpine treatment on the specific binding of DHA in rat pineal gland. Male 3-month-old Fisher rats were treated chronically with saline or reserpine (0.25 to 4 μmoles/kg, i.p.) once daily for 3 days and killed 24 hr after the last dose. One group of rats was killed 30 min after acute treatment with a single 4 μmoles/kg dose of reserpine. Pineal glands were homogenized and specific DHA binding was determined. Each bar represents the mean binding (± SEM) of the number of experiments shown in parentheses in each bar. Asterisks (*) indicate statistically significant differences in specific DHA binding in reserpine-treated rats vs that found in saline-treated animals (**p <0.01; ***p <0.001). Binding in saline-treated rats was 520 ± 40 fmoles DHA bound/mg protein. (Taken from ref. 28).

($p < 0.001$) increase in receptor density (B_{max} = 220 ± 3 vs 300 ± 12 fmoles/mg protein in saline and reserpine-treated cortices, respectively). These findings indicate that the reserpine-induced increase in the responsiveness of brain adenylate cyclase to norepinephrine may be due, at least in part, to an increase in the receptor component of the β-receptor-adenylate cyclase complex.

To determine whether aged animals could exhibit the same adaptive response to reserpine as young rats, we compared specific DHA binding in various brain tissues from 3- and 24-month-old rats following chronic reserpine treatment. The results showed that aged tissue had an impaired ability to increase the number of adrenergic receptors in response to reserpine treatment i.e., in comparison

with the reserpine response in young rats, the response in aged rats was decreased in cerebral cortex and abolished in cerebellum. We also found that reserpine was much more lethal to the aged rats than to the young animals; whereas the young rats tolerated daily doses as high as 8 μmoles/kg, i.p., for several days, 50 to 75% of 24-month-old rats died after 2 days of treatment with doses of 2 or 4 μmoles/kg, i.p. Increased side effects also have been noted in aged humans following reserpine treatment[49]. The results suggest that certain brain areas from senescent rats may have an impaired capacity to increase receptor density in response to the reserpine-induced reduction of noradrenergic input. An impairment in this compensatory mechanism, in fact, may explain the loss of receptors found in the aging brain. The increased lethality of reserpine in the aged rats may also represent a decreased ability of these animals to compensate for the decline in central and peripheral sympathetic nervous activity produced by this drug.

b. Effect of Trifluoperazine Treatment on β-Adrenergic Receptors in Rat Brain. For several years our laboratory has studied the interactions of neuroleptic drugs with the various components of the catecholamine receptor-adenylate cyclase-cyclic nucleotide phosphodiesterase system in an attempt to identify the neuroleptic receptors in brain. We have been particularly interested in understanding what actions chronic neuroleptic treatment may have on this system with the hope of elucidating the mechanisms by which these drugs produce their therapeutic and side effects. Much emphasis in the literature has been given to the effects of these agents on decreasing dopaminergic neuronal transmission in the basal ganglia and limbic forebrain[50,51]. While interference with dopaminergic function very likely explains the extrapyramidal side effects produced by these drugs, there is as yet no definitive proof that this action also is responsible for their therapeutic effects, for it is well known that these drugs can also inhibit the central effects of other catecholamines such as norepinephrine[39]. For example, neuroleptic drugs have been reported to inhibit norepinephrine-sensitive adenylate cyclase in various brain areas[37-39]. The extent to which these agents may influence central noradrenergic transmission, particularly following long-term use, has been largely ignored, even though such an effect may account for some of their side effects such as sedation and hypotension and may even be important in their therapeutic action. Therefore, we studied the effect of chronic trifluoperazine treatment on DNA binding in various areas of the rat brain, since changes in the density of β-adrenergic receptors appear to be a reliable reflection of the level of central noradrenergic transmission.

Rats were treated with trifluoperazine (1 μmole/kg, s.c.) once daily for 2 weeks and sacrificed 24 hours after the last dose. Figure 2 shows that trifluoperazine caused a significant increase in specific DNA binding in the cere-

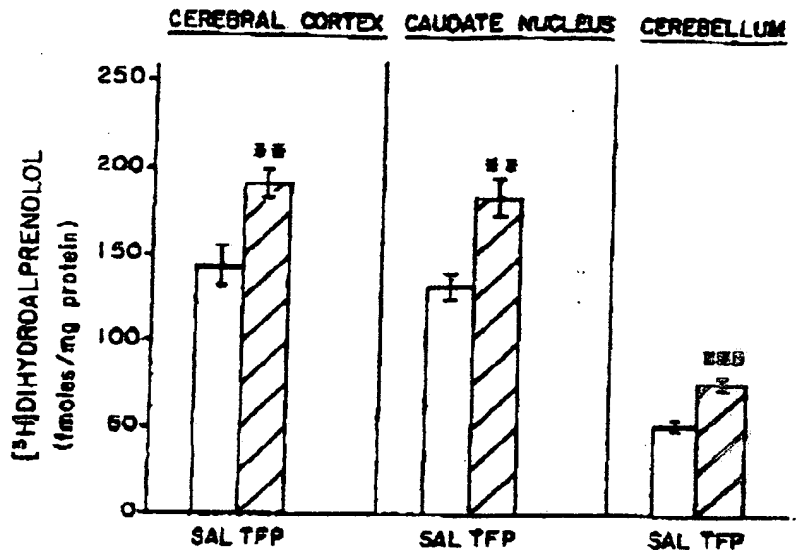

Figure 2. Effect of chronic trifluoperazine treatment on the specific binding of DHA in rat brain. Male Sprague Dawley rats were treated chronically with saline or trifluoperazine (1.0 μmole/kg, s.c) once daily for 2 weeks and killed 24 hr after the last dose. The cerebral cortex, caudate nucleus and cerebellum were homogenized and specific DHA binding was determined. Each bar represents the mean binding (± SEM) of six experiments. Asterisks(*) indicate statistically significant differences in specific DHA binding in trifluoperazine-treated vs saline-treated rats (**p< 0.01; ***p <0.001). Sal = saline;TFP=trifluoperazine.

bral cortex, corpus striatum and cerebellum. These data suggest that chronic trifluoperazine treatment,by reducing noradrenergic transmission, caused a compensatory increase in β-adrenergic receptors. A direct effect of trifluoperazine on the DHA binding assay *per se* is an unlikely explanation for the increase in specific binding since high concentrations of trifluoperazine actually decrease DHA binding *in vitro* (IC_{50} = 100 μM).

Trifluoperazine may interfere with noradrenergic transmission in brain by at least three mechanisms: 1) by preventing the release of norepinephrine from nerve endings; 2) by inhibiting the action of norepinephrine by blocking post-junctional β-adrenergic receptors; 3) by preventing the action of the endogenous protein modulator, which is purported to modify the activity of adenylate cyclase[52,53]. Studies in our laboratory have shown that neuroleptic drugs inhibit the effects of the modulator protein by binding specifically to it[54,55].

In fact, this action of neuroleptics may be the mechanism by which these drugs inhibit catecholamine-sensitive adenylate cyclase, although a direct inhibition of the catecholamine receptors cannot be ruled out.

The present findings of an increased density of β-receptors in rat brain following chronic treatment with trifluoperazine may explain some of the long term clinical actions of these drugs. For example, if the sedation and hypotension produced by these drugs is, in fact, related to interference with central noradrenergic transmission, then a compensatory increase in β-receptors may explain the reported development of tolerance to these side effects[56]. In addition, one must consider the possibility that the therapeutic effects of these agents may also be due to a chronic supersensitivity of central noradrenergic mechanisms. In this regard, Stein and Wise[57] observed that the behavioral deficits found in schizophrenic patients resembled those produced in animals by 6-hydroxydopamine (6-OHDA), a neurotoxin that destroys catecholaminergic neurons. They proposed that schizophrenia may result from the abnormal production of a 6-OHDA-like neurotoxin that destroys noradrenergic nerves in certain brain areas (positive reward centers) and leads to the manifestations of the disease. Therefore, the induction of β-adrenergic receptors and noradrenergic supersensitivity produced by chronic neuroleptic treatment could compensate for a loss of noradrenergic function and lead to clinical improvement.

SUMMARY

The aging process is associated with a reduced number of β-adrenergic receptors in several areas of the rat brain; there is no change, however, in the affinity of these receptors for adrenergic antagonists. Furthermore, compared with brain tissue from young rats, aged rats also show an impaired ability to increase receptor density in response to decreased sympathetic input. On the other hand, tissues from aged rats can respond to increased sympathetic input by reducing their number of receptors. The finding of an impaired capacity of brain tissue from aged rats to develop receptor supersensitivity in response to reduced noradrenergic input may explain the decline in β-adrenergic receptors with age and the reduced responsiveness of aged tissue to adrenergic stimuli.

The findings that chronic treatment of rats with DRI produces a decreased density of β-adrenergic receptors in brain, whereas reserpine and trifluoperazine treatment increases receptor density suggests that one may have to reevaluate the current theories of the mechanism of action of these compounds. In fact, the chronic alterations in the density of β-adrenergic receptors induced by psychoactive agents may provide a more rational explanation for their therapeutic action and may provide the biochemical reason for the development of tolerance to certain of their effects.

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